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A QUANTITATIVE ANALYSIS OF LOCAL ANAESTHETIC ALTERATION OF MINIATURE END-PLATE CURRENTS AND END-PLATE CURRENT FLUCTUATIONS

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SUMMARY

1. The effect of the local anaesthetic QX222 on the kinetics of miniature end-plate currents (m.e.p.c.s) and acetylcholine (ACh) induced end-plate current (e.p.c.) fluctuations was studied in voltage-clamped frog cutaneous pectoris neuromuscular junctions made visible with Nomarski differential interference contrast optics.

2. In Ringer solution the m.e.p.c.s decayed with a single exponential time course and the e.p.c. fluctuation spectra were characterized by single Lorentzian functions, with the spectral cut-off frequency well predicted by the m.e.p.c. decay rate.

3. In the presence of 0·1–0·5 mM QX222 at −50 to −100 mV holding potential both the e.p.c. fluctuation spectrum and the m.e.p.c. decay consisted of a fast and a slow component, with the cut-off frequency of each spectral component predicted by the decay rate of the corresponding constituent of the m.e.p.c.

4. Hyperpolarization increased the decay rate and relative amplitude of the fast component of the m.e.p.c. and decreased the decay rate of the slow m.e.p.c. component.

5. With 0·05 mM QX222 and −70 mV holding potential the m.e.p.c.s and e.p.c. fluctuation spectra consisted of three components. The third component of the m.e.p.c. and e.p.c. spectra had nearly the same decay rate and cut-off frequency as was found at the same end-plate under equivalent conditions before drug exposure.

6. The kinetic predictions of four different schemes for local anaesthetic action were compared with the observed m.e.p.c.s and e.p.c. fluctuations.

7. Schemes in which the local anaesthetic acted by creating two

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kinetically distinct populations of acetylcholine receptors or by interacting with the ACh receptor to produce a biphasic exponential decay of the end-plate channel conductance did not accurately predict the e.p.c. fluctuation spectrum.

8. The variance of the e.p.c. fluctuations vanished at the reversal potential indicating that local anaesthetic action was not due to the presence of different ion selective end-plate channels.

9. QX222 action could be explained by alteration of the ACh receptors such that they sequentially change from one conductance state to another. A specific case in which QX222 binds to the ACh receptors in its open state creating a partially blocked state, was found to be the most parsimonious.

10. The conductance, γ, of a single end-plate channel was estimated from e.p.c. fluctuations. In Ringer's solution $\gamma = 24{\cdot}4 \pm 1{\cdot}2$ (S.D.) pmho. In the presence of 0·1 mM to 0·5 mM QX222 the effective single channel conductance, $\bar{\gamma}$, varied from 14·2 to 1·39 pmho.

11. $\bar{\gamma}$ decreased with increased local anesthetic concentration, hyperpolarization, or decreased temperature. The variation in $\bar{\gamma}$ is thought to reflect the dependence on the experimental conditions of the relative probability that the ACh receptors is in an open *vs* a partially blocked state.

Introduction

Normally end-plate currents (e.p.c.s) at the frog neuromuscular junction decay with a single exponential time course (Magleby & Stevens, 1972*a*; Kordaš 1972*a*, *b*; Gage & McBurney, 1972, 1975; Anderson & Stevens, 1973; Magleby & Terrar, 1975; Dionne & Stevens, 1975; see also Fig. 5 of Maeno, 1966) which may be represented as

$$I(t) = I_0 e^{-\alpha t}, \tag{1}$$

where $I(t)$ is the current t msec after an arbitrary zero time, I_0 is the amplitude and α is the decay rate. In the presence of procaine (Gage & Armstrong, 1968; Kordaš, 1970; Deguchi & Narahashi, 1971; Maeno, Edwards & Hashimura, 1971) or lidocaine and its derivatives (Steinbach, 1968*a*; Beam, 1976*a*) the decay of e.p.c.s and miniature end-plate currents (m.e.p.c.s) have multi-component decay time courses. Usually the e.p.c.s consist of an initial component which decays faster than the e.p.c.s recorded in drug-free solution followed by a prolonged component which decays slower than the control. Beam (1976*a*) showed that the trimethyl derivative of lidocaine, QX222, produces e.p.c.s which decay as the sum of three exponentials:

$$I(t) = \sum_{i-1}^{3} I_i e^{-\alpha_i t}, \tag{2}$$

where the subscripts 1, 2 and 3 refer to the slowly, rapidly and intermediately decaying components. The intermediate component has the same decay rate as control e.p.c.s and is thought to represent a population of unmodified receptors (Beam, 1976*a*).

Constant concentrations of ACh produce fluctuations in the end-plate conductance (Katz & Miledi, 1970, 1971, 1972) due to the opening and closing of end-plate channels. Analysis of these fluctuations provides a means of directly studying the kinetics of end-plate channels. Anderson & Stevens (1973) used e.p.c. fluctuation analysis to demonstrate that the rate of end-plate channel closure, rather than the decay of the cleft concentration of ACh, determined the time course of decay of e.p.c.s and m.e.p.c.s. Several mechanisms have been suggested to explain the complex alteration of e.p.c.s and m.e.p.c.s by local anaesthetics (Maeno, 1966; Steinbach, 1968*b*; Katz & Miledi, 1975; Beam, 1976*b*). The goal of this paper is to discern which scheme best describes the end-plate action of local anaesthetics by mathematically formalizing each proposal or a representative example of the proposal, and then comparing the predicted form of the e.p.c. fluctuation spectrum with the data.

Only those models in which the local anaesthetic causes the end-plate channel to sequentially change from one conductance state to another appear to account for the experimental results. A specific case in which QX222 binds to the ACh receptor in its open state creating a partially blocked state, was found to be the most parsimonious because it was able to account for the experimental evidence without arbitrary specification of any kinetic terms, and was consistent with the proposed mechanism for QX222 blockage of sodium currents in the frog node of Ranvier (Strichartz, 1973).

A preliminary report of some of these findings has been presented (Ruff, 1976).

METHODS

All observations reported in this paper were made on m. cutaneous pectoris obtained from *Rana pipiens* of 2½–3 in body length. Both winter and summer varieties of frog were used. The summer frogs were kept at room temperature in a tank with running cold tap water. Winter frogs were stored at 40–50° F. With respect to the parameters measured in this study, muscle cells from the two types of frogs behaved similarly. The muscle was placed external surface up in a small Lucite chamber of similar construction to that described by Dionne & Stevens (1975) and dissected down to a single layer. The dissection technique resembles that of Dreyer & Peper (1974; see also Blioch, Glagoleva, Lieberman & Nenachev, 1968; Dionne & Stevens, 1975). During experiments the muscle was bathed in frog Ringer solution with the composition (mM): NaCl 117·2; KCl, 2·5; $CaCl_2$, 1·8; Na_2HPO_4, 3·16; and NaH_2PO_4, 0·85; to which 100 nM tetrodotoxin (Sigma) had been added. QX222 (Astra) was added to the Ringer from a frozen stock solution of 50 mM to produce

the desired drug concentration of 0·05–0·5 mM. Hypertonic QX-Ringer solution was made by adding sufficient sucrose to the previous solutions to bring the concentration to 2·5 M sucrose. The pH of all solutions was adjusted to 7·2.

The preparation in its chamber was placed on the stage of a modified Zeiss–Nomarski microscope described by Dionne & Stevens, 1975; (see also McMahan, Spitzer & Peper, 1972). Experiments were carried out using a Zeiss × 16 objective with a working distance of ~ 0·4 mm and × 10 or × 15 eyepieces; this allowed clear visualization of the end-plates and micro-electrodes. Temperature was controlled by regulating the current flowing through two Peltier coolers with a potentiometer. Thermal gradients were minimized by filtering the light source to eliminate infrared, by placing a $\frac{1}{16}$ in. thick rectangular piece of silver between the chamber and the coolers, and by locating the coolers symmetrically under the two ends of the chamber. A small hole was milled in the silver to allow free movement of the microscope condenser. A small thermistor was placed near the myoneural region to monitor temperature.

The two-electrode voltage clamp used has been described (Magleby & Stevens, 1972*a*; Dionne & Stevens, 1975) and is similar to that introduced by Takeuchi & Takeuchi (1959). Fibres at least 40 μm in diameter with end-plate regions containing an isolated nerve terminal of < 200 μm length were selected and impaled with two glass micro-electrodes filled with 3 M-KCl, one for passing current, the other for measuring potential. The electrodes were located near the middle of the end-plate and were separated by ~ 20 μm to minimize clamp distortion and to maximize the uniformity of voltage control of the end-plate. Low impedance voltage and current electrodes (1 – 3 MΩ) were used to reduce thermal noise in the voltage clamp and to improve the clamp's frequency response. Clamp gain was typically $\geqslant 10^4$. A third micro-electrode for iontophoresis, filled with ~ 2·5 M-ACh chloride (Sigma) was positioned extracellularly 35–50 μm above the clamp electrodes to reduce the possibility of local fluctuations in ACh concentration. In some experiments a micropipette containing hypertonic QX-ringer was located 5 μm above the centre of the end-plate.

The iontophoretic technique (Nastuk, 1953) is similar to that detailed by Dionne & Stevens (1975). Iontophoretic electrodes had, when measured in saline, resistance in the range 20–80 MΩ. Electrodes with unstable resistances were discarded. Backing currents of 15–25 nA were used. Backing was considered adequate if the iontophoretic electrode could be moved to within 1–2 μm of a voltage-clamped end-plate without producing detectable e.p.c.s due to the leakage of ACh. The feed-back circuit for maintaining constant iontophoretic current has been described by Dionne & Stevens (1975).

Because rapidly changing end-plate currents were studied in these experiments it was important to quantify the amount of capacitance between the electrodes and ground and the interelectrode capacitance. The extent of electrode-ground capacitive coupling was measured by placing an electrode in the bath and measuring the amount of current that flowed through the electrode when driven by a constant magnitude sinusoidal voltage. The measured current did not vary appreciably with frequency from 0·5 Hz to 2·5 kHz. The amount of interelectrode capacitance was measured by comparing the potential measured by the voltage electrode, in response to a sinusoidal current of fixed amplitude applied to the current electrode, when both electrodes were in a cell and when the voltage electrode was barely withdrawn from the fibre. For frequencies from 0·5 Hz to 2·5 kHz the potential recorded when the voltage electrode was extracellular was less than 2 % of that measured when both electrodes were intracellular. Two factors probably contributed to minimize stray capacitance: firstly, the level of the bath was kept near the muscle surface (~ 0·3 mm) to reduce

electrode-bath capacitive coupling; secondly, the microscope objective located between the two clamp electrodes was grounded so that it acted like a shield to minimize the inter-electrode capacitance.

The technique of e.p.c. fluctuation recording and analysis is similar to that described by Anderson & Stevens (1973). In this study two channels of e.p.c. fluctuations were converted on-line to digital form at sampling intervals of 0·5 or 1 msec and stored on magnetic tape with a PDP-11/20 computer. One channel contained a low gain (× 100 mV/nA) signal. The other channel was a high gain (× 2000 mV/nA) filtered (Krohn-Nite model 3700 band pass filter set for Butterworth response between 1 and 500 Hz for the 1 msec sampling interval or between 2 and 1000 Hz for the 0·5 msec sampling interval, 40 db/decade rolloff) signal. Usually sixteen records of 1·024 sec duration (0·512 sec duration for 0·5 msec sampling interval) were obtained for each cell at a given set of experimental conditions. The high gain records were screened to exclude those containing m.e.p.c.s and were processed by a fast Fourier transform routine (Anderson & Stevens, 1973) to obtain the e.p.c. fluctuation spectra. Slight attenuation of the high and low frequency components of the spectra due to the bandpass filter was compensated for. To minimize contributions from background noise which is not correlated with the ACh produced current fluctuations, control spectra (obtained in the absence of ACh) were subtracted from the e.p.c. fluctuation spectra. In practically all cases this correction amounted to at most a few per cent. The spectra were fit by a computer generated curve consisting of the sum of two or three Lorentzians in the form of eqn. (4). The cut-off frequencies of the generated curve were determined by the decay rates of the corresponding components of the m.e.p.c., and the amplitudes of the Lorentzians were adjusted by eye to best fit the e.p.c. fluctuation spectra. To minimize contamination of the data due to desensitization, 'noise' collection intervals were separated from each other and from periods of m.e.p.c. collection by several minutes.

The equilibrium potential was determined as the membrane potential at which e.p.c.s produced by short iontophoretic pulses of ACh reversed from inward to outward current as the cell was further depolarized. To change bathing solutions the cell being studied was unclamped and the chamber was emptied of the original solution and refilled with the new bathing solution. The bathing solution was exchanged 10–15 times. After the preparation had returned to its original temperature the cell under study was reclamped.

M.e.p.c.s were collected with the aid of the computer in a manner similar to Dionne & Stevens (1975). Due to high frequency noise in the voltage clamp (see description of voltage clamp noise on p. 622 of Anderson & Stevens, 1973) it was necessary to filter the m.e.p.c.s (5–10 kHz low pass Bessel filter, 40 db/decade roll-off). To improve the signal to noise ratio and speed data analysis several m.e.p.c.s from a given end-plate were averaged using the computer. The decay time courses of the m.e.p.c.s were averaged, as shown in Fig. 1, by scaling each m.e.p.c. to a fixed amplitude, then aligning the decay phase of a given m.e.p.c. with that of the averaged m.e.p.c. The mean amplitude of the latter could then be calculated using the average of the inverse of the amplitude scaling factors. As seen in Fig. 1 the process of aligning the decay phases of the m.e.p.c. often resulted in a 'smearing' of the rising phase of the averaged m.e.p.c. (see Gage & McBurney, 1972). Usually ten or more m.e.p.c.s from a given cell were averaged. There were only two criteria for not including a m.e.p.c. in the average: (1) if the m.e.p.c. had an unstable base line; (2) if the m.e.p.c. had a greatly attenuated and slowed initial component. Such m.e.p.c.s constituted less than 5 % of the total population of m.e.p.c.s and probably arose from distant end-plates on the muscle fibre.

The accuracy of the method of m.e.p.c. averaging was checked by individually

analysing the m.e.p.c.s and comparing the average of the analysis of the population of m.e.p.c.s with the analysis of the averaged m.e.p.c. In five end-plates for which this was done the two methods agreed to within 10 % for the rate constants and the amplitudes of the components of the m.e.p.c. In addition, m.e.p.c.s evoked by localized application of hypertonic QX-Ringer solution by pressure ejection from a micropipette (Fatt & Katz, 1952; Furshpan, 1956; Dionne & Stevens, 1975) had the same average amplitude and time course as those which occurred spontaneously. This indicated that the spontaneously sampled m.e.p.c.s were not significantly distorted by membrane cable properties. The selection of small (200 μm) end plates undoubtedly contributed to the uniformity of m.e.p.c.s by reducing cable attenuation which would have been most serious for the initial fast components of the m.e.p.c. (Gage & McBurney, 1972).

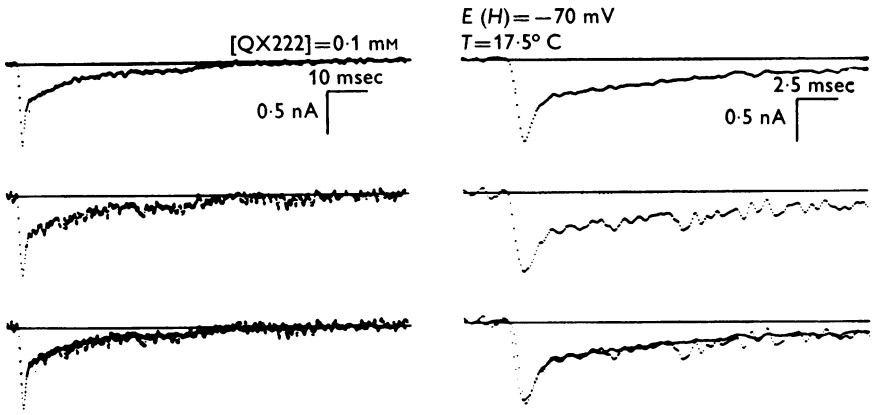


Fig. 1. Illustration of the technique for averaging m.e.p.c.s. The top pair of traces are the average of fourteen m.e.p.c.s. The middle pair of records show a raw m.e.p.c. which has been multiplied by 0·95 and shifted 10 μsec along the time axis so that its decay coincides with the decay of the averaged m.e.p.c. The bottom set of traces are the superposition of the averaged and raw m.e.p.c. Inward currents exhibited as downward deflexions.

The analysis of the amplitudes and rate constants of the components of the averaged m.e.p.c.s was carried out in a manner similar to that of Beam (1976*a*) and is illustrated in Fig. 2. A computer-generated exponential curve with variable amplitude and time constant was adjusted to fit the slowest component of the m.e.p.c. The generated curve was subtracted from the m.e.p.c. and the process was repeated for the next component.

RESULTS

QX222 causes the m.e.p.c.s and e.p.c. fluctuation spectra to have multiple components

The dramatic effect of QX222 on the time course of the average m.e.p.c. and e.p.c. fluctuation spectra can be seen by comparing a cell's behaviour before and after applying the drug. Cells in Ringer solution have averaged

m.e.p.c.s with single exponential decays in the form of eqn. (1) (Magleby & Stevens, 1972*a*; Kordaš, 1972*a*, *b*; Gage & McBurney, 1972, 1975; Anderson & Stevens, 1973; Magleby & Terrar, 1975; Dionne & Stevens, 1975; see also Fig. 5 of Maeno, 1966). The insets in Fig. 3 illustrate the

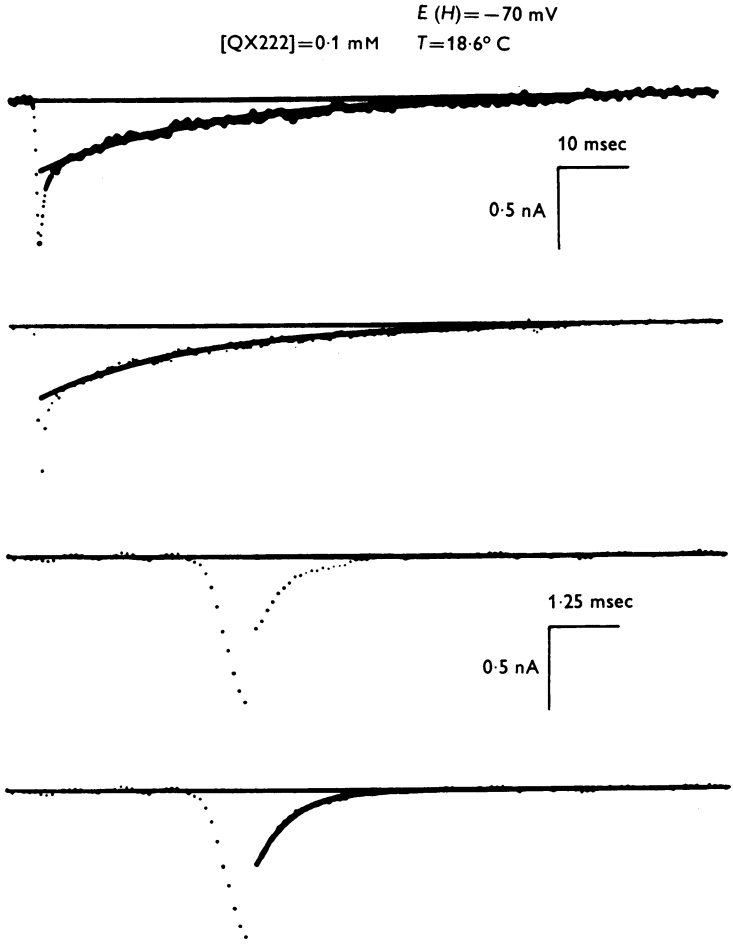


Fig. 2. Decompositon of an averaged m.e.p.c. in the form of eqn. (2). The top trace shows a computer generated exponential whose amplitude and decay rate have been adjusted to align to the slow phase of the m.e.p.c. The computer curve is more clearly seen in the subsequent trace in which only every fourth point of the m.e.p.c. is displayed. The computer curve is then subtracted from the m.e.p.c. and the remaining fast component of the m.e.p.c. is shown in the second trace from the bottom. The superposition of the initial component of the m.e.p.c. decay and a computer generated exponential which has been aligned to the remainder of the m.e.p.c. is shown in the bottom trace.

form of the m.e.p.c.s recorded in Ringer solution. The e.p.c. fluctuation spectra for these cells are nicely fitted by a single Lorentzian function

$$S(f) = A/(1+(f/f_c)^2), \tag{3}$$

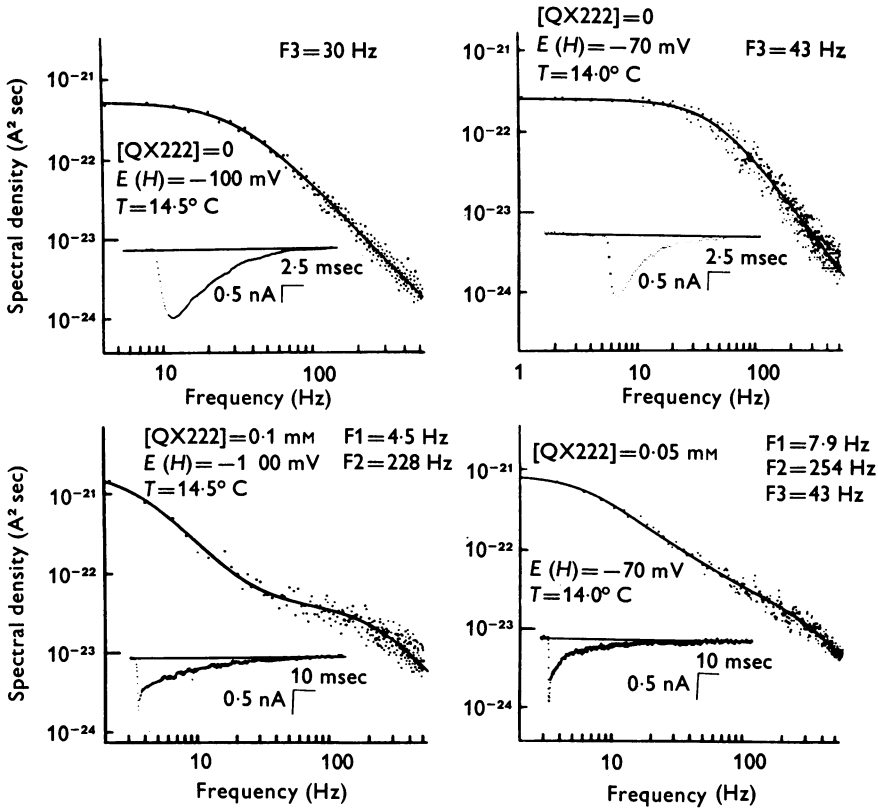


Fig. 3. Comparison of the averaged m.e.p.c. and e.p.c. fluctuation spectrum before and after the application of QX222. The upper data records were obtained from two different cells before the application of local anaesthetic. The lower records were obtained from the same cells following the application of QX222 in the concentrations indicated. The ordinate is a logarithmic axis of spectral density in units of A^2. sec. The continuous line through the spectral points is a theoretical spectrum of the form of eqn. (3) for Ringer solution, or of the form of eqn. (4) in the presence of local anaesthetic. The averaged m.e.p.c. is shown below the spectrum. The cut-off frequency, f, of each spectral component is determined from the decay rate, α_i, of the corresponding component of the m.e.p.c. by the relationship $f_i = \alpha_i/2\pi$. The spectral cut-off frequencies to the nearest Hz for f_2 and f_1, and to the nearest tenth Hz for f_1 are shown in the upper right corner of each spectrum. The ratio of the spectral amplitudes A_2/A_1, was equal to 0·0305 for the cell on the right and 0·0119 for the cell on the left. Eqn. (A10) predicted that A_2/A_1 would equal 0·0283 for the cell in the right and 0·0127 for the cell on the left.

where $S(f)$ is the spectral density at frequency f, A is the amplitude of the Lorentzian, and f_c is the cut-off frequency of the spectrum. In accord with Anderson & Stevens (1973) $f_c = \alpha/2\pi$. The exponential decay of the averaged m.e.p.c., the form of the spectra, and the relationship between the decay rate of the m.e.p.c. and cut-off frequency of the spectra are predicted by Magleby & Stevens' (1972*b*) quantitative model for end-plate channel kinetics. According to this scheme once ACh has bound to the ACh receptor the receptor-agonist complex can fluctuate between two conformations; one corresponding to an 'open' or conducting end-plate channel, the other to a 'closed' or non-conducting channel.

Following the application of QX222 both the m.e.p.c., and the e.p.c. 'noise' spectra show multiple components. As demonstrated by Beam (1976*a*; see also Fig. 5 of Maeno, 1966) it is possible to describe the complex in m.e.p.c. decay as a sum of exponential decays in the form of eqn. (2). The e.p.c. spectra can be fit by a sum of Lorentzian functions

$$S(f) = \sum_{i=1}^{3} A_i/(1 + (f/f_i)^2), \tag{4}$$

where A_i is the amplitude and f_i the cut-off frequency of each component with $f_1 < f_3 < f_2$. Again the cut-off frequencies of the spectra were well predicted by the relationship $f_i = \alpha_i/2\pi$. That the decay rates of the averaged m.e.p.c. continue to reflect the underlying e.p.c. gating kinetics strongly supports the hypothesis that local anaesthetic alteration of e.p.c. kinetics results from an interaction with the ACh receptor which alters the end-plate channel closing kinetics (Steinbach, 1968*a*, *b*; Katz & Miledi, 1975; Beam, 1976*b*). For concentrations of QX222 of 0·1 mM or greater and membrane potentials more negative than -50 mV, the m.e.p.c.s spectra could both be accurately characterized by two components. Some of the cells shown in Figs. 3 and 4 demonstrate two-component m.e.p.c.s and e.p.c. fluctuation spectra.

Two cells studied at 0·05 mM QX222 and -70 mV holding potential were characterized by three-component m.e.p.c.s and spectra. On close examination of the averaged m.e.p.c. of the cell shown in Fig. 3, which was studied at 0·05 mM QX222, one notes that following the initial rapid decay there is a period of intermediate decay which is succeeded by a phase of very slow decay of the m.e.p.c. For this cell $S(f)$ did not decline as rapidly for $f_1 < f < f_2$ as would a two-component spectrum due to the presence of the intermediate component. The portion of the spectrum of this cell between f_1 and f_2 could not be as well fitted by a two-component spectrum. For the two cells studied at 0·05 mM QX222, the rate constant of decay of the intermediate phase of the averaged m.e.p.c. was nearly equal to the pre-drug m.e.p.c. decay rate. The data support Beam's (1976*a*) observation

that the decay rate of the intermediate phase of the e.p.c. equalled the 'normal' decay rate, and that the amplitude of the intermediate component decreases relative to that of the fast and slow components as one increases the local anaesthetic concentration.

In addition to changing the m.e.p.c. and e.p.c. fluctuation kinetics, local anaesthetics also decrease the peak amplitude of the m.e.p.c. (Furakawa, 1957; Maeno, 1966; Gage & Armstrong, 1968; Steinbach, 1968*a*; Hirst & Wood, 1969; Kordaš, 1970; Deguchi & Narahashi, 1971; Maeno, Edwards & Hashimura, 1971; Katz & Miledi, 1975; Beam, 1976*a*) as shown in Fig. 3. As will be discussed later, QX222 was found to decrease the effective conductance of an end-plate channel. The equilibrium potential of all cells before and after the application of local anaesthetic was within 3 mV of 0 mV. This agrees with previous studies, indicating that local anaesthetics do not alter the equilibrium potential of the e.p.c.s (Deguchi & Narahashi, 1971; Maeno *et al.* 1971; Beam, 1976*a*).

Hyperpolarization increases the decay rate and relative amplitude of the initial m.e.p.c. component and slows the decay of the prolonged component

Both the rate constants and relative amplitudes of the fast and slow components of the averaged m.e.p.c. vary systematically with holding potential. Due to cell to cell variation, the relationship is best seen by studying the same cell at different voltages. As illustrated by the cell in Fig. 4 following a hyperpolarizing step in membrane potential, the slow component of both the m.e.p.c. and spectrum became slower and the fast component of both became faster. In addition, after hyperpolarization the amplitude of the rapid component of the m.e.p.c. increased relative to that of the slow component. This voltage dependence of the rate constants and relative amplitudes of the components of the m.e.p.c. was found in the seven cells, shown in Table 1, studied at different holding potentials. Following changes in membrane potential, the rate constants of the averaged m.e.p.c. continued to predict the cut-off frequencies of the corresponding components of the spectrum. The voltage dependence of local anaesthetic action found in this study is similar to that previously reported (Steinbach, 1968*a*; Kordaš, 1970; Beam, 1976*a*).

Brief summary of the models for local anaesthetic action

Several mechanisms have been proposed to account for local anaesthetic alteration of the e.p.c. The proposals will be discussed briefly, and a representative case of each of the suggested schemes will then be formalized in order to compare its predictions for the behaviour of the m.e.p.c. and e.p.c. fluctuation spectrum with the data. Since the majority of data were collected from cells with two-component m.e.p.c.s and spectra, only theories to

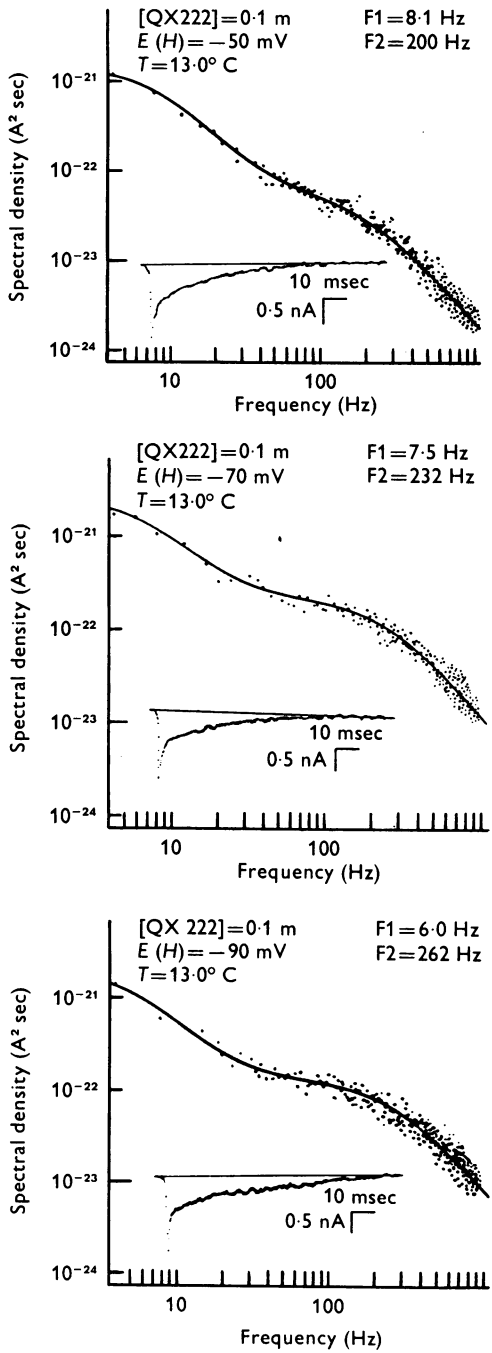


Fig. 4. Illustration of the voltage dependence of QX222 action. A_2/A_1 = 0·0180 at −50 mV, 0·0430 at −70 mV, and 0·0329 at −90 mV; eqn. (A10) predicted that A_2/A_1 would equal 0·0180 at −50 mV, 0·0394 at −70 mV, and 0·0302 at −90 mV. All data records are from cell 4 in Table 1. Consult Fig. 3 for further details.

account for the fast and slow phases of the m.e.p.c. and e.p.c. 'noise' will be considered. The significance of the intermediate or 'normal' phase will be discussed later.

TABLE 1. Voltage dependence of the decay rates and relative amplitudes of the m.e.p.c. components in the presence of QX222

Cell no.	[QX222] (mM)	V (mV)	T (°C)	M.e.p.c. rate constants (msec^{-}) α_2	α_2	I_1/I_2
1	0·1	−70	13·0	0·0466	1·30	0·841
		−90	13·0	0·0414	2·01	1·07
2	0·1	−70	10·0	0·0411	1·20	0·714
		−90	10·0	0·0345	1·57	1·00
3	0·1	−50	11·0	0·0545	1·06	0·583
		−100	11·0	0·0281	1·30	0·732
4	0·1	−50	13·0	0·0507	1·25	0·445
		−70	13·0	0·0472	1·46	1·22
		−90	13·0	0·0380	1·64	1·31
5	0·5	−50	16·0	0·0396	1·39	0·517
		−70	16·0	0·0311	1·63	1·00
6	0·5	−50	13·0	0·0384	1·27	0·652
		−70	13·0	0·0296	1·55	1·36
7	0·5	−70	17·0	0·0215	1·21	0·752
		−100	17·0	0·0187	1·76	1·00

Four mechanisms have been proposed to explain local anaesthetic action at the end-plate. If the end-plate channel can only exist in discrete conductance states, then there are two possible ways in which local anaesthetics may act. Firstly, the drug may act by creating two parallel populations of receptors with different end-plate channel closing rates (Beam, 1976*b*; Gage, 1976). Alternately, the drug may interact with the ACh receptor so that the receptor changes sequentially from one conducting state to another (Steinbach, 1968*b*; Adams, 1975; Beam, 1976*b*). A third scheme assumes that end-plate channel conductance is not restricted to discrete states; the local anaesthetic interacts with the ACh receptor so that the end-plate channel conductance decays with a double exponential time course, similar to the decay of the m.e.p.c. (Katz & Miledi, 1975). According to a fourth proposal there are separate end-plate channels for Na and K ions, and local anaesthetics act by differentially changing the time course of the conductance of the two pathways (Maeno, 1966; Gage & Armstrong, 1968; Deguchi & Narahashi, 1971; Maeno *et al.* 1971).

Data analysis indicates that in the presence of local anaesthetics: (1) there is only one class of ion selective end-plate channel; (2) the end-plate channel

exists in only discrete conductance states; and (3) the drug acts by causing the receptor to change from one conducting state to another.

A specific case of the sequential class of models in which QX222 binds to the open state of the ACh receptor creating a partially blocked state is the most parsimonious because it accurately predicts the form of the spectra based upon the m.e.p.c. without any arbitrary specification of rate constants.

In the following sections the various proposals for local anaesthetic alteration of end-plate kinetics will be formalized, and the predicted behaviour of the m.e.p.c.s and ACh induced e.p.c. fluctuation will be compared with the data.

Kinetic predictions of a specific case of the sequential model

Steinbach (1968*b*) and more recently Adams (1975) proposed that local anaesthetics alter e.p.c.s according to a kinetic scheme in which the drug binds to the receptor-transmitter complex in its open configuration and produces a transmitter-receptor-local anaesthetic complex with a much lower single channel conductance. For concentrations of local anaesthetic and membrane depolarizations at which the e.p.c. consists of only two components, a kinetic scheme which will account for the action of a local anaesthetic, in this case QX222, may be represented as

$$n\mathrm{T}+\mathrm{R}\underset{k_2}{\overset{k_1}{\rightleftharpoons}}\mathrm{T}_n.\mathrm{R}\underset{A}{\overset{B}{\rightleftharpoons}}\mathrm{T}_n.\mathrm{R}^*+\mathrm{Q}\underset{F}{\overset{G}{\rightleftharpoons}}\mathrm{T}_n.\mathrm{R}^*.\mathrm{Q}, \tag{5}$$

where T represents a molecule of ACh, R signifies the ACh receptor, Q is the local anaesthetic, n is the number of transmitter molecules bound to the receptor, $\mathrm{T}_n.\mathrm{R}$ is the receptor-agonist complex associated with a closed channel, $\mathrm{T}_n.\mathrm{R}^*$ is the open conformation of this complex, and $\mathrm{T}_n.\mathrm{R}^*.\mathrm{Q}$ is the transmitter-receptor-local anaesthetic complex which is assumed to have negligible conductance compared to $\mathrm{T}_n.\mathrm{R}^*$; k_1, k_2, A, B, G, and F are the rate constants for the processes indicated. In accord with Magleby & Stevens (1972*b*) it is assumed that the conformation changes are rate limiting and that agonist concentration is far from a saturating level.

This scheme predicts (consult appendix for details) a double exponential decay of m.e.p.c.s in the form of eqn. (2) with observed rate constants

$$\alpha_1 = (A+F+G.\mathrm{Q}-((A+F+G.\mathrm{Q})^2-4A.F)^{\frac{1}{2}})/2$$

and

$$\alpha_2 = (A+F+G.\mathrm{Q}+((A+F+G.\mathrm{Q})^2-4A.F)^{\frac{1}{2}})/2$$

with $\alpha_2 > \alpha_1$. The predicted spectra will have two components with cut-off frequencies $f_1 = \alpha_1/2\pi$. If G increases and F decreases with membrane hyperpolarization, then α_1 and α_2 may have the voltage and concentration dependence of the experimentally observed rate constants. The predicted relative amplitude of the m.e.p.c. components is $I_2/I_1 = (\alpha_2.F)/(F.\alpha_1)$. Given the voltage and local anaesthetic concentration dependence of α_2 and α_1,

this relationship qualitatively predicts the observed relative increase in the amplitude of the fast component of the m.e.p.c. with hyperpolarization and increased local anaesthetic concentration.

Example of a parallel model

Gage (1976) proposed that local anaesthetics may alter e.p.c. kinetics due to a dual effect on the ACh receptor. Local anaesthetics may increase the fluidity of the end-plate membrane. Accordingly, the rapid decay phase would result from a population of ACh receptors which is associated with a membrane of high fluidity. Local anaesthetic might slow the rate of channel closure of other ACh receptors by directly binding to them. This 'parallel' scheme may be represented as

$$\begin{array}{ccc} n\mathrm{T}+\mathrm{R} \underset{k_2}{\overset{k_1}{\rightleftharpoons}} \mathrm{T}_n.\mathrm{R} \underset{\alpha_2}{\overset{\beta_2}{\rightleftharpoons}} \mathrm{T}_n.\mathrm{R}^* \\ \overset{+mQ}{\underset{k_3}{\downarrow\uparrow}} k_4 \\ n\mathrm{T}+\mathrm{R}.\mathrm{Q}_m \underset{k_6}{\overset{k_5}{\rightleftharpoons}} \mathrm{T}_n.\mathrm{R}.\mathrm{Q}_m \underset{\alpha_1}{\overset{\beta_1}{\rightleftharpoons}} \mathrm{T}_n.\mathrm{R}^*.Q_m, \end{array} \qquad (6)$$

where m is the number of local anaesthetic molecules binding to the receptor; and k_{1-6}, α_{1-2}, and β_{1-2} are the rate constants for the indicated processes, each of which may vary with local anaesthetic concentration as well as voltage.

This model predicts (consult Appendix for details) double exponentially decaying m.e.p.c.s with rate constants α_2 and α_1 where $\alpha_2 > \alpha_1$. The spectra will consist of two phases with cut-off frequencies $f_1 = \alpha_1/2\pi$.

The specific sequential model accurately predicts the spectral behaviour whereas the parallel model does not

Comparison of m.e.p.c. and spectra recorded from the same cell provides a direct means of comparing the predictions of the kinetic schemes above with the data. For scheme (5) the ratio of the relative amplitudes of the spectral and m.e.p.c. components is:

$$(A_2/A_1)/(I_2/I_1) = \alpha_1/\alpha_2 \qquad (\mathrm{A}\,10)$$

whereas scheme (6) predicts:

$$(A_2/A_1)/(I_2/I_1) = (\alpha_1/\alpha_2)^2.\gamma_2/\gamma_1, \qquad (\mathrm{A}\,14)$$

where γ_2 and γ_1 are respectively the single channel conductance of $\mathrm{T}_n.\mathrm{R}^*$ and $\mathrm{T}_n.\mathrm{R}^*.\mathrm{Q}_m$ in scheme (6).

The kinetic predictions of a generalized sequential model in which the conductance of $\mathrm{T}_n.\mathrm{R}^*.\mathrm{Q}$ is not constrained to be small compared to the conductance of $\mathrm{T}_n.\mathrm{R}$ are similar to those of kinetic scheme (5), with eqn. (A 10) containing a multiplier term consisting of a combination of the rate constants and single channel conductances associated with the model. The

predictions of other members of the 'parallel' class of models are similar to those of kinetic scheme (6).

In scheme (6) the local anaesthetic alters only the rates of conformation change and not the conformation themselves. Anderson & Stevens (1973) and C. Lewis (unpublished results) noted only slight variation in the single channel conductance of normal receptors, and found that single channel conductance did not vary significantly with temperature or holding potential. Colquhoun, Dionne, Steinbach & Stevens (1975) found that the average single channel conductance varied with the agonist used, but for a given agonist there was little variation in single channel conductance among different cells. Therefore, γ_2/γ_1 should not depend significantly on voltage. Accordingly, scheme (6) and other members of the parallel class of models predict that the ratio of the relative amplitudes of the spectral and m.e.p.c. components would vary as the square of the ratio of the decay rates of the m.e.p.c. components, whereas scheme (5) predicts a linear relationship. By varying the experimental conditions it was possible to study cells with different values of α_1/α_2 and thus determine which scheme best described the data.

Twenty-five cells were studied at 0·05, 0·1, and 0·5 mM QX222, at holding potentials of -50 to -100 mV, and at temperatures of 10–18·6° C. Several cells were studied at two or more holding potentials. Fig. 5 illustrates that $(A_2/A_1)/(I_2/I_1)$ varied linearly with α_2/α_1. eqn. (A 10) predicted the relationship between the ratio of the amplitudes of the spectral components A_2/A_1, and the m.e.p.c. components I_2/I_1 to within 10% of the observed values for all the cell studied. Therefore, kinetic scheme (6) and other members of the parallel class of models cannot explain the end-plate action of QX222 if, as argued earlier, the ratio of the single channel conductances, γ_2/γ_1, for the parallel model is independent of voltage and drug concentration. By similar reasoning Katz & Miledi (1975) have also argued that local anaesthetics do not act by creating two parallel populations of receptors.

Exponential variation of single channel conductance does not accurately predict the spectral form

In the two previous models it was assumed that the end-plate channel could exist in only a finite number of conductance states when activated by a given agonist. Katz & Miledi (1975) considered the possibility that the multi-component 'ACh noise' spectra recorded from end-plates in the presence of procaine might result from a double exponential decay of end-plate channel conductance of parallel form to the decay of m.e.p.c.s. For this model of local anaesthetic action the current from a single end-plate channel, $I_u(t)$, may be represented as

$$I_u(t) = M(I_1 e^{-\alpha_1 t} + I_2 e^{-\alpha_2 t})\, U(t), \tag{7}$$

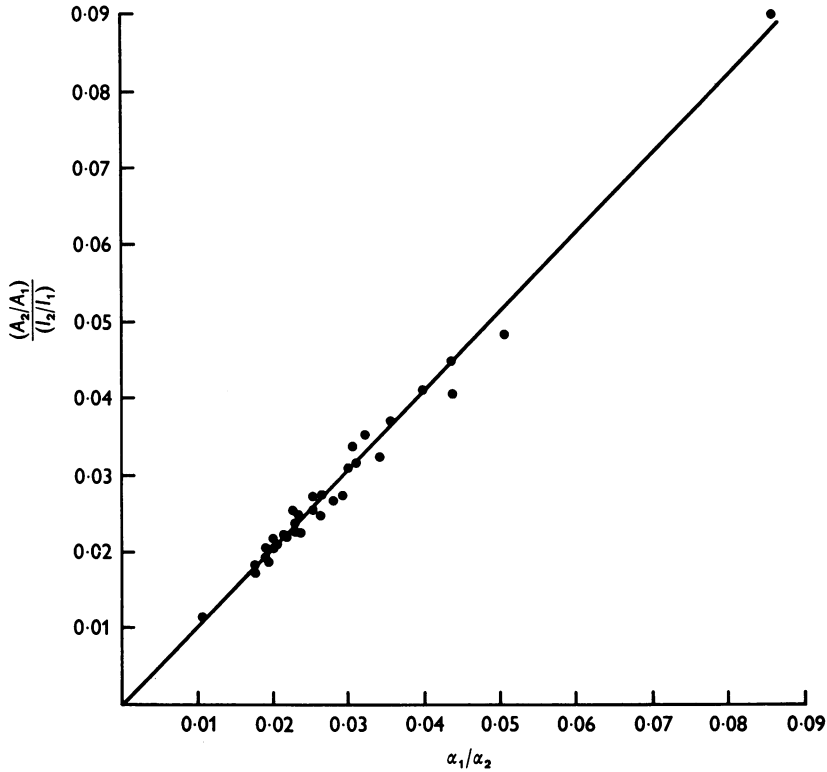


Fig. 5. Dependence of the ratio of the relative amplitudes of the spectral and m.e.p.c. components on the ratio of the slow and fast decay rates of the m.e.p.c. for all the end-plates studied in the presence of QX222. The continuous line is the relationship predicted by eqn. (A10). The least-square linear regression line for the thirty-three data points had a slope of 0·986 and intercepted the ordinate at 0·0002.

where M is a scaling factor, $U(t)$ is the unit step function, i.e. $U(t < 0) = 0$ and $U(t \geqslant 0) = 1$, and I_1, I_2, α_1, α_2 are respectively the amplitudes and rate constants of the slow and fast components of the averaged m.e.p.c. The spectra can be calculated directly from this model (Rice, 1944):

$$S(f) = 2\psi|F\{I_u(t)\}|^2, \tag{8}$$

where ψ is the frequency of occurrence of the individual end-plate currents, $||^2$ indicates that the Fourier transformed current, $F\{I_u(t)\}$, is multiplied by its complex conjugate. The result is

$$S(f) = L\left[\frac{1}{\alpha_1^2(1+(2\pi f/\alpha_1)^2)} + \frac{(I_2/I_1)^2}{\alpha_2^2(1+(2\pi f/\alpha_2)^2)} + \frac{2(I_2/I_1)\,(1+(2\pi f)^2/(\alpha_1.\alpha_2))}{\alpha_1.\alpha_2(1+(2\pi f/\alpha_1)^2)(1+2(\pi f/\alpha_2)^2)}\right], \tag{9}$$

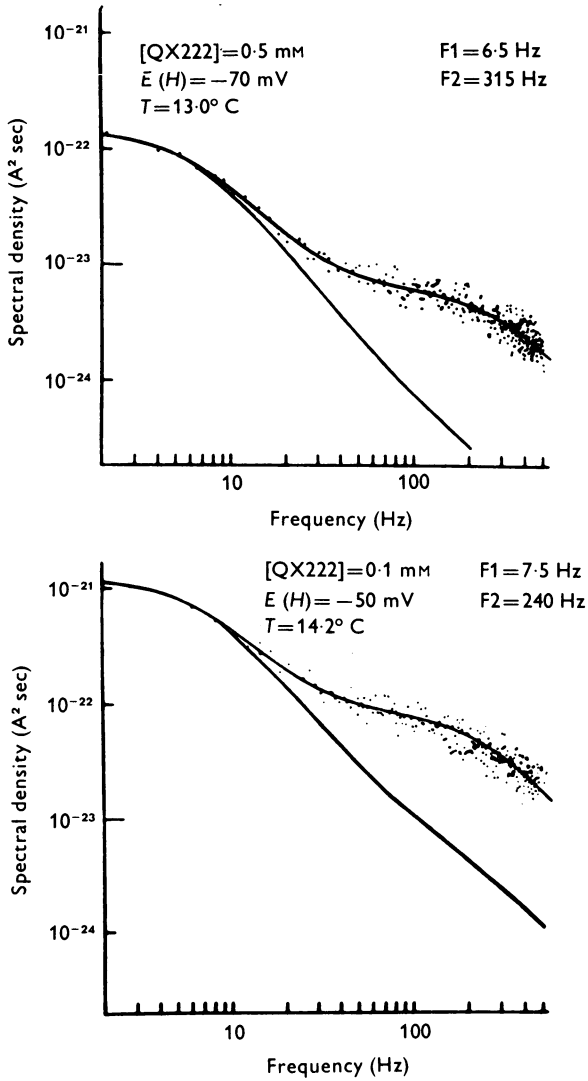


Fig. 6. Comparison of the spectral predictions of the specific case of the sequential model, kinetic scheme (5), with the predictions of the model assuming exponential decay of end-plate conductance, scheme (7) in two different cells. For each cell the upper curve through the data points is the spectrum predicted by kinetic scheme (5) which was calculated from the averaged m.e.p.c. using eqns. (4) and (A10). The lower curve, which departs from the spectral data at higher frequency, is the prediction of scheme (7) obtained from eqn. (9). Consult Fig. 3 legend for further details.

where $L = 2M^2I_1^2\psi$. Therefore, using the amplitudes and rate constants of the averaged m.e.p.c. and $S(O)$ from the observed spectra, one can directly calculate the spectra predicted by a double exponential decay of end-plate channel conductance. In Fig. 6 the spectral predictions of kinetic schemes (5) and (7) were compared to the spectra of two cells. The spectra predicted by the double exponential decay of end-plate conductance does not describe the data as well as that predicted by the specific sequential model. Both kinetic schemes accurately predict the spectrum for $f \leqslant f_1$. Above the first cut-off frequency eqn. (9) begins to depart from the data because of a faster high frequency roll-off of predicted spectral intensity. For all the cells studied at 0·1 and 0·5 mM QX222 there was usually an order of magnitude difference between the actual spectral intensity at 500 Hz and that predicted by eqn. (9). Based on these observations, local anaesthetic action cannot be attributed to the production of end-plate channels with two phase exponential decay of conductance.

QX222 action is not the result of separate ion-selective end-plate channels

Maeno (1966) initially proposed that there exist separate end-plate channels for Na and K and that local anaesthetic alteration of e.p.c.s was due to a selective kinetic effect on the end-plate pathway for Na. Kordaš (1969, 1970) and Magleby & Stevens (1972*a*, *b*) questioned the ability of the two channel hypothesis to account for the monotonic voltage dependence of the e.p.c. decay rate and the existence of a definite reversal potential for normal or drug altered e.p.c.s. Examination of the voltage dependence of the variance of ACh produced e.p.c. fluctuations provides a direct test for the existence of separate ionic pathways at the end-plate. If two or more independent populations of ion selective end-plate channels exist (having discrete or continuous conductance states) with different equilibrium potentials, then one should find an appreciable amount of e.p.c. current fluctuation when ACh is applied at the effective reversal potential. Conversely, if all the ions pass through a single class of end-plate channel there should be no appreciable e.p.c. variance when ACh is applied at the reversal potential since no net current will flow through any channel which is open. For the two-channel model we may represent the iontophoretically produced e.p.c. as:

$$I = \Delta g_{Na}(V - V_{Na}) + \Delta g_K(V - V_K) = N_S(\gamma_{Na}P_{Na}(V - V_{Na}) + \gamma_K P_K(V - V_K)), \tag{10}$$

where Δg_{Na} and Δg_K are respectively the increase in Na and K conductance produced by ACh, V_{Na} and V_K are the equilibrium potentials of the postulated Na and K end-plate channels, N_S is the number of ACh receptors exposed to the steady concentration of ACh, γ_{Na} and γ_K are respectively

the single channel conductance for Na and K and P_{Na} and P_K are respectively the normalized probability that a Na or K channel will be open. The variance of the e.p.c. as a function of voltage, $\sigma^2(V)$ is

$$\sigma^2(V) = N_S \cdot (\gamma_{Na}^2 P_{Na}(V - V_{Na})^2 + \gamma_K^2 P_K (V - V_K)^2). \quad (11)$$

From eqn. (11) it is clear that the e.p.c. variance predicted by a two-channel model will never vanish provided that the equilibrium potentials of the two channels are not equal. To gain a qualitative idea of the voltage dependence of σ^2 let us assume that γ_{Na}, γ_K, P_{Na} and P_K are relatively constant from -50 mV to the observed reversal potential, ~ 0 mV. Assuming $V_K = -100$ mV and $V_{Na} = +50$ mV, eqn. (10) predicts $\gamma_{Na} P_{Na} / \gamma_K P_K = 2$. The variance is then

$$\sigma^2(V) = N_S \gamma_K P_K (2\gamma_{Na}(V - V_{Na})^2 + \gamma_K (V - V_K)^2.$$

Accordingly, the ratio of the e.p.c. variance measured at -50 mV compared to that at the reversal potential will vary from 0·43 to 3·4 for γ_{Na}/γ_K between 0·1 and 10.

TABLE 2. Comparison of the variance of e.p.c. fluctuations at -50 mV and the reversal potential

Cell	[QX222]	Holding potential (mV)	R.m.s. 'Noise' (nA) Ionto	R.m.s. 'Noise' (nA) Control	Net variance ((nA)²)
3	0·1 mM	−50	0·185	0·022	$2{\cdot}66 \times 10^{-2}$
		+2	0·0150	0·0140	1×10^{-6}
5	0·5 mM	−50	0·206	0·012	$3{\cdot}76 \times 10^{-2}$
		+1	0·0125	0·0128	—
6	0·5 mM	−50	0·265	0·011	$6{\cdot}4 \times 10^{-2}$
		−2	0·0132	0·0130	4×10^{-8}
8	0·1 mM	−50	0·243	0·021	$4{\cdot}93 \times 10^{-2}$
		−3	0·0192	0·0190	4×10^{-8}

The voltage dependence of e.p.c. variance was studied in four cells at 0·1 or 0·5 mM QX222. For each cell the same intophoretic current was used to stimulate e.p.c. fluctuations at -50 mV and the reversal potential. The results shown in Table 2 indicate that there was no appreciable 'ACh noise' at the reversal potential. The net e.p.c. variance at the reversal potential was more than four orders of magnitude smaller than the net variance recorded at -50 mV. Similar results are found for cells in normal Ringers (Dionne & Ruff, 1976). Although Anderson & Stevens (1973) did not specifically discuss the single channel conductance in the critical range of ± 20 mV holding potential, if their finding that the single channel conductance recorded in Ringer solution did not vary significantly with holding potential can be extrapolated to the reversal potential, then their data can

be taken as evidence against the existence of separate end-plate channels for different ions.

These results indicate that there are not independent ion-selective channels with different equilibrium potentials at the end-plate. If Ca passes through the proposed Na-selective end-plate channel (Deguchi & Narahashi, 1971) the argument is not substantially changed since the combined Na- and Ca-selective channel would have a different reversal potential than the K-selective pathway. The data do not rule out the possibility that there are separate ion selective end-plate channels with a common gating mechanism. Since such an arrangement would function as a single population of end-plate channels permeable to both ionic species, it is more parsimonious to envision Na and K currents being conducted through a single pathway.

Effective single channel conductance

The specific sequential model, represented by kinetic scheme (5), adequately accounts for local anaesthetic alteration of the m.e.p.c.s. In deriving the relationship between the amplitudes of the m.e.p.c. and spectral components predicted by this model, eqn. (A 10), it was assumed that the conductance of the drug bound complex, $T_n.R^*.Q$, was small compared with that of the agonist-receptor complex, $T_n.R^*$. While it is not possible at present to measure directly the individual conductances of a multistate system, the range of variation of the effective single channel conductance of end-plates in the presence of QX222 indicates that the conductance of $T_n.R^*$, γ_1, is at least an order of magnitude greater than the conductance of $T_n.R^*.Q$, γ_2.

For cells studied in normal Ringer solution the single channel conductance γ, is (Stevens, 1972; Anderson & Stevens, 1973):

$$\gamma = \frac{\sigma^2}{\mu_I(V - V_{eq})}, \tag{13}$$

where μ_I is the mean e.p.c. γ may also be determined from the spectra (Anderson & Stevens, 1973)

$$\gamma = \frac{S(0)\,\alpha}{4\mu_I(V - V_{eq})}. \tag{14}$$

Note that eqn. (14) was modified to account for the one sided spectra, $f \geqslant 0$, used in this study (see pp. 81–84 of Bendat & Piersol, 1971). The mean single channel conductance determined by eqn. (73) for ten cells in normal Ringer was $24{\cdot}4 \pm 1{\cdot}2$ pmho (S.D.); one of the cells was studied at two holding potentials. This value is consistent with the Colquhoun *et al.* (1975) value of 25·0 pmho for the mean end-plate channel conductance with ACh as the

agonist. The single channel conductance estimated from the amplitude of the spectrum, eqn. (14), agreed well with the estimates of γ from 'noise' variance, eqn. (13).

Consideringthe specific case of the sequential model, the end-plate channel can exist in more than one conducting state in the presence of local anaesthetic. The single-channel conductance determined by eqn. (13) is then an effective conductance, $\bar{\gamma}$, which reflects not only γ_1 and γ_2 but also the probability that the receptor will be in conformation $T_n.R^*$ or $T_n.R^*.Q$. The effective conductance is a mean of γ_1 and γ_2 weighted according to the amount of time the receptor spends in the corresponding states. The relationship between $\bar{\gamma}$, γ_1 and γ_2 as determined by eqn. (13) is:

$$\bar{\gamma} = \frac{\gamma_1^2 P_1 + \gamma_2^2 P_2}{\gamma_1 P_1 + \gamma_2 P_2}, \tag{15}$$

where P_1 and P_2 are the steady-state probabilities that the receptor is respectively in state $T_n.R^*$ or $T_n.R^*.Q$. The variance was obtained by evaluating the covariance function, eqn. (A3) at $t = 0$. If γ_2 and γ_1 are constant and $\gamma_2 < \gamma_1$, then eqn. (15) predicts that $\bar{\gamma}$ should decrease as P_2/P_1 increases. For the specific sequential model $P_2/P_1 = [Q].G/F$, where [Q] represents the concentration of QX222 and G increases relative to F with hyperpolarization. Consequently, the specific sequential model predicts that $\bar{\gamma}$ should decrease following an increase in local anaesthetic concentration or hyperpolarization. The values of $\bar{\gamma}$ determined by eqn. (13) for cells in 0·1 mM or 0·5 mM QX222 are shown in Fig. 7. The data indicate that, at a given temperature, $\bar{\gamma}$ is lower at a higher drug concentration or larger negative holding potential, in accordance with the expectations of the specific sequential model. From eqn. (A5), for the sequential model of local anaesthetic action, $\bar{\gamma}$ should be related to the amplitudes and cut-off frequencies of the spectra by

$$\bar{\gamma} = \frac{(A_1.\alpha_1 + A_2.\alpha_2)}{4\mu_I(V - V_{eq})}. \tag{16}$$

The effective single channel conductance estimated by eqn. (16) agreed well with the values determined from eqn. (13) for all the cells in 0·1 or 0·5 mM QX222.

In deriving eqn. (A10), which relates the amplitudes of the m.e.p.c. and spectral components, it was assumed that γ_2 was small compared to γ_1. The actual values of γ_2 and γ_1 cannot be determined accurately without knowing the values of the rate constants G and F. Considered in the context of eqn. (15), the range of $\bar{\gamma}$ shown in Fig. 7 suggests that γ_1 is more than an order of magnitude larger than γ_2. However, without specific knowledge of the rate constants in kinetic scheme (5), it is difficult to determine if an

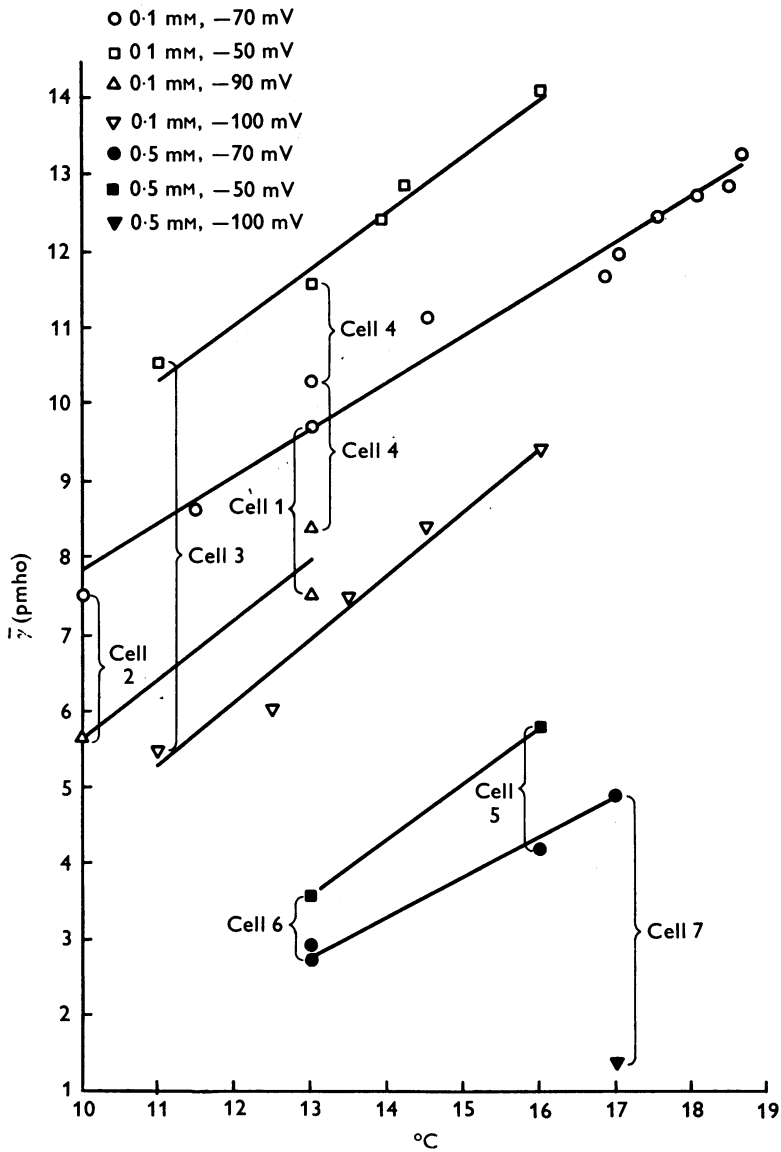


Fig. 7. The dependence of the effective single channel conductance, $\bar{\gamma}$, on temperature at different holding potentials and concentrations of QX222. $\bar{\gamma}$ was calculated by eqn. (13). The symbol identification table is shown in the upper left corner of the graph. The continuous lines are least-square linear regression lines for values of $\bar{\gamma}$ at the same membrane potential and QX222 concentration. The brackets indicate values of $\bar{\gamma}$ from the same end-plate.

order of magnitude difference between γ_1 and γ_2 is sufficiently large to justify the exclusion of terms scaled by γ_2 or γ_2^2 in the derivation of eqn. (A10).

DISCUSSION

The findings of this study confirm and extend evidence that local anaesthetic alterations of e.p.c.s and m.e.p.c.s result from a direct interaction of the drug with the ACh receptor. In addition, this study demonstrates the general utility of 'noise' measurements in discerning underlying membrane conductance gating mechanisms.

Proposed mechanisms of local anaesthetic action

Comparison of m.e.p.c. and e.p.c. fluctuations collected from the same cell provided a means of directly testing several proposed mechanisms for local anaesthetic action at the end-plate. Gage, McBurney & Schneider (1975) suggested that local anaesthetics might alter m.e.p.c.s by changing the fluidity or dielectric constant of the end-plate membrane. One interpretation could be that local anaesthetics increase the fluidity of certain regions of the end-plate membrane, while increasing the dielectric constant of other regions. Provided that the ACh receptor can exist in only discrete conductance states, the previous interpretation of local anaesthetic action would result in a kinetic scheme similar to (6) with the same predicted relationship for the ratio of the relative amplitudes of the spectral and m.e.p.c. components as eqn. (A 14). In general, any kinetic scheme in which the receptors are segregated into two functionally independent or 'parallel' populations before, or at the time of, agonist binding will predict a relationship between the amplitudes of the m.e.p.c. and spectral components which differs from eqn. (A 14) by only a multiplicative factor. Eqn. (A 14) did not accurately predict the relationship between the spectral and m.e.p.c. amplitudes; thus the above interpretation of local anaesthetic action does not appear to apply at the end-plate. The proposal could be modified so that local anaesthetic modification of the end-plate membrane would result in a continuous distribution of end-plate channel closing rates. In this case the predicted e.p.c.s and m.e.p.c.s probably could not be decomposed into discrete components. In addition, the e.p.c. fluctuation spectrum of a population of ACh receptors continuously graded end-plate channel closing rates would not display individual components, but would resemble (Halford, 1968; Verveen & DeFelice, 1974):

$$S(f) = A_0 f^{-\phi},$$

where $0 \leqslant \phi \leqslant 2$, with the value of ϕ determined by the relative number of receptors associated with a given end-plate channel lifetime. Thus, it appears that local anaesthetic alteration of e.p.c. kinetics is not primarily

due to changing end-plate membrane properties such as fluidity or dielectric constant.

A second model suggested by Katz & Miledi (1975) assumes that in the presence of local anaesthetic the end-plate channels do not exist in discrete conductance states. On this view, the end-plate channel conductance decays with a double exponential time course of similar form to the decay of m.e.p.c.s. The spectral predictions of this model did not agree with the observed spectra. Aside from the failure of this particular model with continuously varying end-plate channel conductance, other findings suggest that the end-plate channel probably exists in only discrete conductance states. Anderson & Stevens (1973) indicated that protein conformation changes are well described by schemes in which the molecule rapidly switches between distinct states; and that materials such as Gramacidin A and excitability inducing material produce discrete conductance increases in artificial membranes. Studying K channel gating in the frog node of Ranvier, Begenisich & Stevens (1975) concluded that the potassium channel probably only exists in discrete conductance states. Recently, Neher & Sakmann (1976) have recorded extracellular current from small patches of voltage clamped extrajunctional denervated frog muscle membrane, and directly confirmed that ACh induced channels exist in only one open state with a conductance of 24 pmho.

The production of two-component m.e.p.c. and e.p.c. fluctuation spectra by local anaesthetics was best explained by kinetic scheme (5) in which the receptor–agonist complex fluctuates between three conductance states: non-conducting, conducting, and partially conducting or blocked; in addition, the relative transfer rate from the conducting to the blocked state is increased by increasing local anaesthetic concentration and hyperpolarization of the membrane.

A more general version of the sequential scheme, (A 1), considered in the Appendix can also account for the data but would require arbitrary specification of a number of rate constants.

Local anaesthetics may act before and after agonist binding

In the present study the local anaesthetic appears to act both before and after ACh binds to the ACh receptor. We will first consider the initial action of the local anaesthetic. Beam (1976*a*) pointed out that under the appropriate conditions e.p.c.s recorded in the presence of QX222 contained an intermediate component which decayed at the same rate and with the same voltage dependence as e.p.c.s recorded in Ringer solution. The rate constant of the intermediate phase of the e.p.c. did not vary with local anaesthetic concentration. Based on the similarity between the intermediate component of QX222 altered e.p.c.s and normal e.p.c.s he concluded

that the intermediate component represented a population of ACh receptors which had not interacted with the local anaesthetic. Beam (1976*a*) argued that the local anaesthetic must interact with the ACh receptor before agonist binding to account for the graded loss of the normal component of the e.p.c. with increased local anaesthetic concentration of hyperpolarization. Consequently, there could be two groups of receptors; the unaltered receptors would contribute the normal component of e.p.c. decay, while the local anaesthetic altered ACh receptors would produce the fast and slow portions of the e.p.c. Thus to account for the intermediate phase of the m.e.p.c.s and spectra kinetic scheme (5) should be modified to

$$\begin{array}{ccccc} n\mathrm{T}+\mathrm{R} & \underset{k_4}{\overset{k_3}{\rightleftharpoons}} & \mathrm{T}_n.\mathrm{R} & \underset{\alpha}{\overset{\beta}{\rightleftharpoons}} & \mathrm{T}_n.\mathrm{R}^* \\ {\scriptstyle k_5}^{+mQ}\downarrow\uparrow{\scriptstyle k_6} & & & & \\ n\mathrm{T}+\mathrm{R}_1 & \underset{k_2}{\overset{k_1}{\rightleftharpoons}} & \mathrm{T}_n.\mathrm{R}_1 & \underset{A}{\overset{B}{\rightleftharpoons}} & \mathrm{T}_n.\mathrm{R}_1^*+\mathrm{Q} \underset{F}{\overset{G}{\rightleftharpoons}} \mathrm{T}_n.\mathrm{R}_1^*.\mathrm{Q}, \end{array} \qquad (17)$$

where R represents the unaltered receptors, and R_1 the local anaesthetic modified receptors. If the initial interaction of the charged local anaesthetic molecule with the ACh receptor is voltage dependent, then scheme (17) could account for the decrease in the relative amplitude of the intermediate component of the e.p.c. with increase in local anaesthetic concentration or hyperpolarization (Beam, 1976*a*, *b*). Kinetic scheme (17) reduces to scheme (5) for conditions at which the vast majority of receptors are in the drug altered state. That local anaesthetic interaction with the ACh receptor can alter the receptor's properties is suggested by the observation of Weber & Changeux (1974) that local anaesthetics non-competitively alter the affinity of ACh receptor for agonist and decrease the rate of binding of α-neurotoxin (see also Cohen, Weber & Changeux, 1974). It has been proposed that atropine (Lapa, Albuquerque & Daly, 1974; Adler & Albuquerque, 1976) and histrionicotoxin (Albuquerque, Barnard, Chiu, Lapa, Dolly, Jansson, Daly & Witkop, 1973; Albuquerque, Kuba & Daly, 1974) alter e.p.c.s by interacting with the ACh receptor at a site distinct from the agonist binding site. The work of Foidart & Gridelet (1974) and Poste, Papahadjopoulos, Jacobs & Vail (1975) indicates that local anaesthetics may alter the properties of specific membrane proteins.

In addition to the initial binding, in schemes (17) and (5) the local anaesthetic also interacts with the altered ACh receptor after the agonist has bound and the end-plate channel has opened. Three possible mechanisms for this interaction will be discussed. Firstly, the local anaesthetic may bind to the receptor and induce a conformation change from the open to the block conformation. The voltage dependence of the rate constants G and F could then result from a dipole moment difference between the open and

blocked states (Magleby & Stevens, 1972*b*; Gage & McBurney, 1975) Alternately, the local anaesthetic may bind to a site inside the end-plate channel. For this mechanism the rate of end-plate channel blockage would increase with local anaesthetic concentration or membrane hyperpolarization (Strichartz, 1973; Woodhull, 1973; Courtney, 1975); however, in this case the rate of blocking and unblocking could be affected by the amplitude of the e.p.c. (Armstrong, 1966, 1969, 1971; Armstrong & Hille, 1972). A third mechanism by which local anaesthetics could interact with the ACh receptor is by perturbing the electric field in its vicinity. Steinbach (1968*a*) demonstrated that the charged form of the local anaesthetics are responsible for altering e.p.c.s. The charged local anaesthetic molecules appear to bind to acidic phospholipids (Skou, 1954; Shanes & Gershfield, 1960; Ohki, 1970; Papahadjopoulos, 1970; Muller & Finkelstein, 1972; Seeman, 1972, Papahadjopoulos, Jacobsen, Poste & Shepard 1975), and thereby change the membrane surface electrical potential (Muller & Finkelstein, 1972). Local anaesthetic binding to acidic phospholipids could change the electric field experienced by the ACh receptor in the same manner as hyperpolarization of the membrane. In this case local anaesthetic action might be modified by substances such as Ca^{2+} which affect the membrane surface electrical potential (Ohki, 1970; Papahadjopoulos, 1970; McLaughlin, Szabo & Eisenman, 1971; Muller & Finkelstein, 1972; Papahadjopoulos *et al.* 1975). The temperature dependence of $\bar{\gamma}$ shown in Fig. 7 indicates that, provided γ_1 and γ_2 from scheme (5) are not temperature dependent, the relative probability that a receptor is in the unblocked *vs.* blocked conformation, P_1/P_2, increases with temperature. This could result from a binding energy associated with removal of a local anaesthetic molecule from a binding site in the end-plate channel or on the surface membrane. Alternately a relatively large activation energy associated with the conformation change from state $T_n . R^* . Q$ to state $T_n . R^*$ would also account for the temperature dependence of $\bar{\gamma}$.

Other agents producing two-component end-plate channel kinetics

There are a number of agents which will alter e.p.d. kinetics. These are detailed by Beam (1976*b*) & Steinbach & Stevens (1976). For many of these agents the e.p.c. consists predominantly of a fast phase. Such end-plate currents could result from a kinetic scheme similar to (5) in which the transition rate from the blocked to the open state was slow compared to the other rate constants. Colquhoun *et al.* (1975) noted that the e.p.c. fluctuation spectra produced by the agonists 3-(*m*-hydroxyphenyl)propyltrimethyl ammonium (HPTMA) and 3-phenylpropyltrimethyl ammonium (PPTMA) had both a high and low frequency component. When activated by either of these agonists the effective single channel conductance was significantly

lower than that associated with ACh. Both the two-component e.p.c. fluctuations spectra and the decreased single channel conductance could result if HPTMA and PPTMA blocked open end-plate channels.

Gage *et al.* (1975) reported that m.e.p.c. recorded in the presence of hexanol were biphasic. Since short chain alcohols lengthen e.p.c. decay times (Gage *et al.* 1975) while long chain alcohols have the opposite effect (Gage, McBurney & Van Helden, 1974), the two-component m.e.p.c.s recorded with hexanol probably resulted from two separate processes rather than a second order kinetic scheme.

Validity of assumptions

In deriving the equations predicting the m.e.p.c. and spectral behaviour the agonist concentration was assumed to be below the saturating level as has been done in previous studies (Magleby & Stevens, 1972*b*; Anderson & Stevens, 1973; Gage & McBurney, 1975; Dionne & Stevens, 1975). Anderson & Stevens (1973) concluded that ACh receptor saturation did not occur with distal iontophoresis of ACh which increased end-plate conductance to greater than 3·5 μmho. In this study iontophoretically applied ACh increased end-plate conductance up to 1 μmho in Ringer solution. Assuming that $\sim 10^7$ channels are present at a frog end-plate (Porter, Chiu, Wieckowski & Barnard, 1973; Barnard, Dolly, Porter & Albuquerque, 1975), my single channel conductance estimate of 24 pmho and peak end-plate conductance of 1 μmho would imply that less than 0·4 % of the channels were open at any time during iontophoretic application of ACh. Hence the assumption of a low effective concentration of ACh is internally consistent with the value of γ obtained. At the snake neuromuscular junction, Hartzell, Kuffler & Yoshikami (1975) stated that a single quantum of ACh did not produce post-synaptic saturation.

In deriving the predicted m.e.p.c. response a second assumption was made, that the decay of the cleft concentration of ACh was rapid in comparison to the decay rates of the components of the m.e.p.c. There is little evidence available to directly test this assumption. Beam (1976*b*, see also Magleby & Stevens, 1972*b*) has recently derived a driving function, which is mathematically proportional to the time course of ACh cleft concentration, for nerve evoked e.p.c.s in the presence of QX222. The driving function rose sharply and then decayed with a half-time of $\sim$ 0·18 msec. If the 'driving function' accurately reflects the time course of ACh concentration, then in some instances the fast component of e.p.c.s recorded in the presence of QX222 could be contaminated by the decay of the cleft concentration of ACh. The transmitter concentration probably decreases more rapidly following release of a single quantum than following nerve evoked release of $\sim$ 100 quanta (Katz & Miledi, 1965*a*, *b*; Barrett & Stevens, 1972).

Therefore, the contamination of the fast component of the e.p.c. due to decay of the cleft concentration of ACh is probably less significant for m.e.p.c.s than for nerve evoked e.p.c.s. There may have been contamination of the initial component of the m.e.p.c. decay by the decay of ACh concentration, but the agreement between the cut-off frequency of the fast component of the e.p.c. fluctuation spectrum and the cut-off frequency predicted by the initial phase of the m.e.p.c. suggests that the contamination was not significant. The possibility that the slow component of the e.p.c. spectra arose from local fluctuations of ACh concentration was tested by the same procedures as used by Anderson & Stevens (1973).

The voltage, temperature, and drug concentration dependence of $\bar{\gamma}$ was attributed to variation in the probabilistic weighting of the constants γ_1 and γ_2. This treatment was based on previous work (Anderson & Stevens, 1973; Colquhoun *et al.* 1975) which indicated that the single channel conductance associated with a given agonist did not vary with changes in temperature or holding potential. However, the variation in $\bar{\gamma}$ could be due to voltage, temperature, and local anaesthetic concentration sensitivity of γ_1 and γ_2.

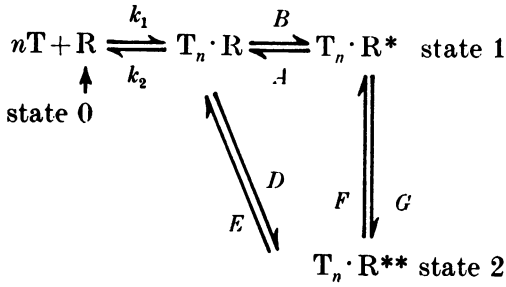
In conclusion, this report indicates that the lidocaine derivative QX222 modifies m.e.p.c.s by altering end plate channel gating. Further, the data are most consistent with the local anaesthetic interacting with the ACh receptor before and after the agonist binds to the receptor.

APPENDIX

This Appendix will deal with the probabilistic treatment of the generalized sequential model, a specific case of the sequential model, and a specific parallel model for local anaesthetic action. For all the kinetic schemes considered in this paper the receptors are assumed to act independently. This does not rule out the possibility that individual receptors are grouped into oligomers and that the oligomer effectively controls the end-plate conductance (Monad, Wyman & Changeux, 1965; see also the recent review by Rang, 1974). In this case the oligomer would represent the 'functional' ACh receptor R in the different schemes. In accord with Magleby & Stevens (1972*b*), it is assumed that the conformation changes are rate limiting and that agonist concentration is far from a saturating level. This implies (i) that the binding and unbinding rate constants, k_1, are large compared to the other rate constants and (ii) that the probability of the receptor being in a state bound with the agonist is relatively small.

General sequential model

The generalized sequential model may be represented as



(A1)

where D and E are the rate constants for the processes indicated; the other symbols are as defined for kinetic scheme (5). The receptor in states R and $T_n.R$ is non-conducting; the states $T_n.R^*$ and $T_n.R^{**}$ have, respectively, conductance γ_1 and γ_2.

Let $P_{ij}(t)$ be the conditional probability that a receptor is in state j at time t given that it was in state i at time $t = 0$, where the subscripts i and j refer to the numbered states represented in eqn. (A1). Considering assumptions (i) and (ii) we have

$$\frac{dP_{i1}(t)}{dt} = -(A+G)P_{i1}(t) + FP_{i2}(t) + BKC(t)^n$$

and

$$\frac{dP_{i2}(t)}{dt} = GP_{i1}(t) - (E+F)P_{i2}(t) + DKC(t)^n, \qquad \text{(A2)}$$

where $K = k_1/k_2$ and $C(t)$ is the time course of the ACh concentration. We can now derive expressions for the e.p.c. fluctuation spectrum in the presence of a steady concentration of ACh and the m.e.p.c.s predicted by scheme (A1).

Spectrum of the sequential model

To calculate the spectra for scheme (A1) it is necessary to solve the eqns. (A2) for the condition that $C(t) = C_s$ (a constant). The co-variance function for the e.p.c. $C_I(t)$, of a single receptor is computed according to:

$$C_I(t) = (V-V_{eq})^2\left[\sum_{i=1}^{2}\sum_{j=1}^{2}(\gamma_i\gamma_j P_{ij}(t)P_i)\right] - (V-V_{eq})^2\left(\sum_{i=1}^{2}\gamma_i P_i\right)^2, \qquad \text{(A3)}$$

where P_i is the unconditional probability that the receptor is in state i, V is the holding potential and V_{eq} is the equilibrium potential. The covariance for the entire population of receptors, N_s, exposed to the steady

concentration of ACh, is obtained by multiplying the co-variance of a single receptor by N_s. For scheme (A1) the covariance for N_s is

$$C_I(t) = \frac{N_s e^{-\alpha_2 t}(V-V_{eq})^2}{\alpha_2-\alpha_1}[\gamma_1^2 P_1(\alpha_2-\beta)+\gamma_2^2 P_2(\alpha_2-\alpha)-\gamma_1\gamma_2(P_1G+P_2F)]$$
$$+\frac{N_s e^{-\alpha_1 t}(V-V_{eq})^2}{\alpha_2-\alpha_1}[\gamma_1^2 P_1(\beta-\alpha_1)+\gamma_2^2 P_2(\alpha-\alpha_1)+\gamma_1\gamma_2(P_1G+P_2F)]$$

where $\alpha = A+G, \beta = E+F, \alpha_1 = (A+E+F+G-((A+E+F+G)^2 - 4.(\alpha.\beta-F.G))^{\frac{1}{2}})/2\,\alpha_2 = (A+E+F+G+((A+E+F+G)^2 - 4.(\alpha.\beta-F.G))^{\frac{1}{2}})/2$, $P_1 = KC_s^n(F.D+\beta.B)/(\alpha_1.\alpha_2)$,

and $P_2 = KC_s^n(G.B+D.\alpha)/\alpha_1.\alpha_2)$. Terms of the form $P_i.P_j$ were dropped due to assumption (*ii*). The one-sided spectral density, $S(f)$, defined for $f \geqslant 0$ is calculated using the Wiener–Khintchine Theorem (cf. Lee, 1960) as

$$S(f) - 2Re[F\{C_I(t)\}],$$

where $Re[\ ]$ indicates the real part of the Fourier transform, $F\{\ \}$. The result is

$$S(f) = \frac{4N_s(V-V_{eq})^2}{\alpha_2-\alpha_1}\left[\frac{\gamma_1^2 P_1(\alpha_2-\beta)+\gamma_2^2 P_2(\alpha_2-\alpha)-\gamma_1\gamma_2(P_1G+P_2F)}{\alpha_2(1+(2\pi f/\alpha_2)^2)}\right.$$
$$\left.+\frac{\gamma_1^2 P_1(\beta-\alpha_1)+\gamma_2^2 P_2(\alpha-\alpha_1)+\gamma_1\gamma_2(P_1G+P_2F)}{\alpha_1(1+(2\pi f/\alpha_1)^2)}\right]. \qquad (A5)$$

For discussion of the covariance function and its relationship to the spectra consult Lee (1960) and Bendat & Piersol (1971) (see also Stevens, 1972, 1975; Verveen & DeFelice, 1974).

M.e.p.c. of the sequential model

Suppose that $C(t)$ in eqn. (A2) results from the release of a single quantum of ACh by the nerve terminal at $t = 0$ and that initially all the receptors are in the unbounded state. The time course of the predicted end-plate current will be given by

$$I(t) = N_I(V-V_{eq})[\gamma_1 P_{01}(t)+\gamma_2 P_{02}(t)], \qquad (A6)$$

where N_I is the number of receptors exposed to the brief pulse of ACh. Eqn. (A2) yields

$$I(t) = \frac{N_I K(V-V_{eq})}{\alpha_1-\alpha_2}\left[(\gamma_1(B(\alpha_1-\beta)-DF)+\gamma_2(D(\alpha_2-\alpha)-BG))\right.$$
$$\times\int_0^t C^n(t-\tau)\,e^{\alpha_2-\tau}\,d\tau + (\gamma_1(B(\beta-\alpha_1)+DF)+\gamma_2(D(\alpha-\alpha_1)+BG))$$
$$\left.\times\int_0^t C^n(t-\tau)\,e^{-\alpha_2\tau}\,d\tau\right].$$

Magleby & Stevens (1972*b*) and Dionne & Stevens (1975), have argued that the time course of decay of the cleft concentration of ACh is very short compared to the decay of nerve evoked e.p.c.s. The decay of ACh concentration following the release of a single quantum should be faster than that for the nerve evoked e.p.c. (Katz & Miledi, 1965*a*, *b*; Barrett & Stevens, 1972). To the extent that the decay of $C(t)$ may be considered as brief compared to the m.e.p.c., the predicted m.e.p.c. is

$$I(t) = \frac{N_I C_I^n (V - V_{eq})}{\alpha_2 - \alpha_1} [(\gamma_1(B(\alpha_2 - \beta) - DF) + \gamma_2(D(\alpha_2 - \alpha) - BG))\, e^{-\alpha_2 \tau} + (\gamma_1(B(\beta - \alpha_1) + DF) + \gamma_2(D(\alpha - \alpha_1) + BG))\, e^{-\alpha_1 \tau}], \quad \text{(A7)}$$

where C_I is the effective peak cleft concentration of ACh.

Special case of the sequential model

Consider a special case of the generalized sequential model for which (1) the rate constants D and E are neligible, (2) state 2 is non-conducting relative to state 1, and (3) the rate constant G depends on the concentration of QX222. These restrictions lead to kinetic scheme (5). The spectrum for this scheme obtained from eqn. (A5) is

$$S(f) = \frac{4N_s(V - V_{eq})^2 \gamma_1^2 P_1}{\alpha_2 - \alpha_1} \left[\frac{\alpha_2 - F}{\alpha_2(1 + (2\pi f/\alpha_2)^2)} + \frac{F - \alpha_1}{\alpha_1(1 + (2\pi f/\alpha_1)^2)}\right], \quad \text{(A8)}$$

where $\alpha_1 = (A + F + G.Q - ((A + F + G.Q)^2 - 4A.F)^{\frac{1}{2}})/2,$

$\alpha_2 = (A + F + G.Q + ((A + F + G.Q)^2 - 4A.F)^{\frac{1}{2}})/2,$

and $P_1 = KC_s^n BF/(\alpha_1 - \alpha_2).$

The predicted m.e.p.c. eqn. (A7) becomes

$$I(t) = \frac{N_I C_I^n (V - V_{eq}) \gamma_1 B}{\alpha_2 - \alpha_1} [(\alpha_2 - F)\, e^{-\alpha_2 t} + (F - \alpha_1)\, e^{-\alpha_1 t}]. \quad \text{(A9)}$$

The ratio of the relative amplitudes of the fast and slow component of the spectra compared to that of the m.e.p.c. is

$$(A_2/A_1)/(I_2/I_1) = \alpha_1/\alpha_2, \quad \text{(A10)}$$

where the components of the m.e.p.c. and spectra have respectively been expressed in the form of eqns. (2) and (4). With arbitrary specification of the rate constants in scheme (A1) the general sequential model will predict the same relationship for the relative amplitudes of the m.e.p.c. and spectra as does this special case.

Example of a parallel model

There are many physical schemes which will result in a 'parallel' model for receptor kinetics, 'parallel' meaning that at any point in time there are

two or more functionally distinguishable populations of receptors that operate essentially independently of each other. Kinetically, this will occur if the states associated with the rate determining conformation changes are separated by fast rate constants, k_i. The spectral and m.e.p.c. predictions for members of the 'parallel' class are similar, therefore only one representative will be discussed. Referring to kinetic scheme (6), if we let state 0 signify R, state 1 represent $T_n.R^*.Q_m$, and state 2 stand for $T_n.R^*$, then states 0, $T_n.R.Q_m$, and $T_n.R.Q_m$ are non-conducting while states 1 and 2 have, respectively, conductance γ_1 and γ_2. Based upon assumptions (i) and (ii) the conditional probabilities for this scheme are

$$\frac{dP_{i1}(t)}{dt} = -\alpha_1 P_{i1}(t) + \beta_1 K_5 \left(\frac{K_3[Q]^m}{1+K_3[Q]^m}\right) C^n(t),$$
$$\frac{dP_{i2}(t)}{dt} = -\alpha_2 P_{i2}(t) + \beta_2 K_1 \left(\frac{1}{1+K_3[Q]^m}\right) C^n(t), \tag{A11}$$

where $K_1 = k_1/k_2, K_3 = k_3/k_4, K_5 = k_5/k_6$, and [Q] is the local anaesthetic concentration. Using eqns (A3) and (A11) the covariance function for this scheme is:

$$C_I(t) = N_s C_s^n \left[\gamma_1^2 \frac{\beta_2 K_5}{\alpha_1}\left(\frac{K_3[Q]^m}{1+K_3[Q]^m}\right) e^{-\alpha_1 t} + \gamma_2^2 \frac{\beta_2 K_1}{\alpha_2}\left(\frac{1}{1+K_3[Q]^m}\right) e^{-\alpha_2 t}\right].$$

From eqn. (A4) the spectrum is

$$S(f) = 4N_s C_s^n (V - V_{eq})^2 \left[\left(\frac{K_3[Q]^m}{1+K_3[Q]^m}\right) \frac{\gamma_1^2 \beta_1 K_5}{\alpha_1^2(1+(2\pi f/\alpha_1)^2)} + \left(\frac{1}{1+K_3[Q]^m}\right)\left(\frac{\gamma_2^2 \beta_2 K_1}{a_2^2(1+2\pi f/\alpha_2)^2}\right)\right]. \tag{A12}$$

The predicted m.e.p.c. decay for this scheme is obtained using eqns. (A6) and (A11)

$$I(t) = N_I C_s^n (V - V_{eq}) \left[\frac{K_3[Q]^m \gamma_1 \beta_1 K_5}{1+K_3[Q]^m} e^{-\alpha_1 t} + \frac{\gamma_2 \beta_2 K_1}{1+K_3[Q]^m} e^{-\alpha_2 t}\right]. \tag{A13}$$

The relationship between the relative amplitudes of the components of the m.e.p.c. and spectra is then

$$(A_2/A_1)/(I_2/I_1) = (\alpha_1/\alpha_2)^2 . \gamma_2/\gamma_1. \tag{A14}$$

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